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The Effects of Chilling in the Light on Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Activation in Tomato (*Lycopersicon esculentum* Mill.)

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Photosynthesis rate, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activation state, and ribulose bisphosphate concentration were reduced after exposing tomato (*Lycopersicon esculentum* Mill.) plants to light at 4°C for 6 h. Analysis of lysed and reconstituted chloroplasts showed that activity of the thylakoid membrane was inhibited and that Rubisco, Rubisco activase, and other soluble factors were not affected. Leaf photosynthesis rates and the ability of chilled thylakoid membranes to promote Rubisco activation recovered after 24 h at 25°C. Thylakoid membranes from control tomato plants were as effective as spinach thylakoids in activating spinach Rubisco in the presence of spinach Rubisco activase. This observation is in sharp contrast to the poor ability of spinach Rubisco activase to activate tomato Rubisco (Z.-Y. Wang, G.W. Snyder, B.D. Esau, A.R. Portis, and W.L. Ogren [1992] Plant Physiol 100: 1858–1862). The ability of thylakoids from chilled tomato plants to activate Rubisco in the assay system was greatly inhibited compared to control plants. These experiments indicate that chilling tomato plants at 4°C interferes with photosynthetic carbon metabolism at two sites, thioredoxin/ferredoxin reduction (G.F. Sassenrath, D.R. Ort, and A.R. Portis, Jr. [1990] Arch Biochem Biophys 282: 302–308), which limits bisphosphatase activity, and Rubisco activase, which reduces Rubisco activation state.

Inhibition of photosynthesis following exposure to low temperatures and high light has been observed frequently in plants of tropical origin (Powles et al., 1983; Martin and Ort, 1985). After a 6-h exposure to 4°C and high light, photosynthesis in tomato at atmospheric CO_2 concentration and 25°C was inhibited by more than 50%, primarily because of direct impairment of chloroplast function (Martin and Ort, 1985; Sassenrath, 1988; Sassenrath and Ort, 1990). The limitation of photosynthesis that persists as plants are rewarmed does not result from any significant loss of photophosphorylation activity (Kee et al., 1986; Wise et al., 1990) but appears to arise from effects on the photosynthetic carbon reduction cycle (Sassenrath, 1988; Sassenrath et al., 1990). Following chilling in the light, the activities of stromal bisphosphatases in tomato were reduced, causing the accumulation of SBP and FBP and a reduced level of RuBP. The bisphosphatases are activated by the Fd/thioredoxin system (Buchanan, 1980), which may be affected by the light-chilling regime. Thus, in tomato, chilling in the light appears to limit the capacity of leaves to regenerate RuBP, the substrate in photosynthetic CO_2 fixation catalyzed by the enzyme Rubisco (EC 4.1.1.39).

Rubisco activity also has been reported to be directly impaired during chilling in the light in short-term (Sassenrath et al., 1990) and long-term (Brüggemann et al., 1992) experiments. After leaves were allowed to recover for 1 h in the dark, Sassenrath et al. (1990) found that Rubisco activity returned to normal. In contrast, Brüggemann et al. (1992) reported a prolonged reduction in the Rubisco activation state that lasted for days. These discrepancies that exist in the literature concerning the effects of chilling in the light on Rubisco activity are perhaps explained by the different experimental procedures used, specifically the experimental chilling temperature, duration of the chill, and light intensity (Sassenrath et al., 1990; Brüggemann et al., 1992; Holaday et al., 1992), and the effect of these different treatments on the various possible mechanisms that might influence Rubisco activity. Light activation of Rubisco is catalyzed by Rubisco activase (Salvucci et al., 1985; Portis et al., 1986) and inhibition of activation could occur from inhibition of Rubisco, Rubisco activase, or some other component.

Recently, light-dependent stimulation of Rubisco activation in lysed spinach chloroplasts was demonstrated. This light requirement, which was in addition to the light required for ATP synthesis (Campbell and Ogren, 1990a), was contingent on electron transport through PSI, the establishment of a pH difference across the thylakoid membrane (Campbell and Ogren, 1990b), and, when ATP, CO_2, Mg^{2+}, and pH were maintained at favorable levels, required Rubisco activase and thylakoid membranes (Campbell and Ogren, 1992). This association between the thylakoid membrane and Rubisco activase could operate to effectively transduce the activation or inactivation of Rubisco in response to changing environmental conditions such as chilling stress.

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Abbreviations: FBP, fructose-1,6-bisphosphate; RuBP, ribulose-1,5-bisphosphate; SBP, sedoheptulose-1,7-bisphosphate.

The objective of this study was to determine whether chilling in the light disrupted the light stimulation of Rubisco activation in the chilling-sensitive species, tomato. Using lysed and reconstituted tomato chloroplasts isolated from light-chilled and recovered plants, we demonstrated the degree of the disruption and subsequent recovery.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Plants of tomato (*Lycopersicon esculentum* Mill. cv. Floramerica) were grown from seed in a controlled environment chamber at 28/25°C (day/night) with a 14-h photoperiod. RH ranged from 60 to 75% and irradiance ranged from 550 to 650 μmol photons m^{-2} s^{-1}. Plants were grown for 3 to 4 weeks in 3-L containers in equal volumes of soil, peat, and perlite and were provided with a complete nutrient solution twice weekly. Plants selected for chilling treatments were placed in a lighted chilling chamber for 6 h with an average irradiance of 700 μmol photons m^{-2} s^{-1} at plant height and a leaf temperature of 4°C. Control plants were not exposed to low temperatures but remained at 28°C in the light. After 6 h, plants were removed from the chamber and placed in a darkened chamber at 25°C and 100% RH for 1 h (chill and control treatment) or for 24 h (recovery treatment). Afterward, plants were returned to the lighted growth chamber for 30 min.

Leaf Photosynthesis

The youngest, fully expanded leaves were selected for measurements of CO_2 assimilation, Rubisco activation, and RuBP. A portion of a leaf (approximately 15 cm^2) was placed in the leaf chamber, and CO_2 uptake was determined in an atmosphere of either 335 ± 12 or 942 ± 18 μL L^{-1} CO_2 using an IRGA in an open system. CO_2-free air and 5% CO_2 were mixed, and flow to the leaf chamber was 0.50 L min^{-1} as controlled by mass flow controllers. Leaf temperature was 25°C as measured with a copper-constantan thermocouple and maintained by a temperature-controlled water bath. Irradiance was 1.5 mmol photons m^{-2} s^{-1}. Humidity of the air entering the chamber was 60%.

Rubisco Activation State and RuBP Levels of Leaf Extracts

Assays of Rubisco activation state were determined on leaves at near atmospheric CO_2. After treatment, leaves were exposed to humidified air (325 μL L^{-1} CO_2, 212 mL L^{-1} O_2) flowing across the upper and lower leaf surface from a manifold with openings 1 cm apart. Flow rate into the manifold was 1.5 L min^{-1} and irradiance at the leaf surface was 600 μmol photons m^{-2} s^{-1}. After 30 min, leaves were immediately plunged into a container of liquid N_2 placed just beneath the leaf. Leaves were ground to fine powder in a mortar and pestle and Rubisco activity was assayed by $^{14}CO_2$ incorporation into acid-stable products as described by Brooks et al. (1988). Rubisco activation state reflects the carbamylation state of Rubisco and was considered to be the initial activity expressed as a percentage of the activity obtained after 15-min activation in the presence of 100 mM Tricine-KOH (pH 8.0), 10 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (0.5 Ci mol^{-1}), and 0.05 mM gluconate-6-P. All reactions were carried out at 25°C.

RuBP concentrations were determined as described by Brooks et al. (1988). After grinding in liquid N_2, the leaf powder was transferred to an Eppendorf[2] tube and 0.50 mL of 3.5% formic acid was added and ground into the frozen powder. After thawing, each tube was centrifuged at 16,000*g* for 4 min and 0.40 mL of the supernatant was transferred to a second Eppendorf tube that contained 1 μL of Kodak2 pH indicator. The supernatant was neutralized by adding 90 to 125 μL of 2 N KOH in 150 mM Tricine-KOH and 10 mM KCl, and the precipitate was removed by centrifugation at 16,000*g*. RuBP concentrations were determined by adding 100-μL aliquots to a Rubisco activity assay containing 100 mM Tricine-KOH (pH 8.0), 10 mM $NaH^{14}CO_3$ (0.5 Ci mol^{-1}), 10 mM $MgCl_2$, and 50 μg of Rubisco. Reactions were incubated for 15 min to ensure conversion of all RuBP present to 3-phosphoglyceric acid.

Preparation of Lysed Chloroplasts

Intact chloroplasts were prepared as described by Robinson and Portis (1988). Turgid leaves (approximately 20 g) were ground in medium A using three 3-s bursts in a Polytron blender. All procedures were carried out at 0°C. Medium A consisted of 330 mM sorbitol, 25 mM Hepes-KOH, 5 mM $MgCl_2$, 10 mM $Na_4P_2O_7$, 4 mM L-ascorbate, 2 mM EDTA, 5 mM DTT, 0.2% (w/v) BSA, and 2% PVP-40 (soluble) adjusted to pH 7.8 with 1 M KOH. $Na_4P_2O_7$, BSA, and ascorbate were made fresh daily and ascorbate and DTT were added just prior to use. After blending, the brei was squeezed through six layers of cheesecloth and two layers of Miracloth and then centrifuged for 1 min at 1500*g* at 4°C in a swing-out rotor. The supernatant was discarded and the pellet containing intact and broken chloroplasts was resuspended in 7.5 mL of medium B consisting of 330 mM sorbitol, 50 mM Hepes-KOH, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 5 mM DTT, 0.2% (w/v) BSA, and 2% (w/v) PVP-40 adjusted to pH 7.8. The resuspension was underlaid with the same medium containing 40% Percoll and centrifuged for 2 min at 1500*g*. Broken chloroplasts formed a layer at the Percoll gradient interface and intact chloroplasts formed a loose pellet. The supernatant was discarded and the pellet resuspended in a small amount of medium B.

Chloroplast intactness was checked visually by phase contrast microscopy and by the ferricyanide intactness assay (Lilley et al., 1975) and were usually 75 to 85% intact. The chloroplast suspension was diluted 10-fold in a buffer consisting of 5 mM Tricine-KOH (pH 8.0) and 20 mM NaCl, 2 mM EDTA, and 10 mM $Na_4P_2O_7$ and sheared repeatedly in a Teflon pestle homogenizer to ensure complete lysis. Lysed chloroplasts were diluted to 0.1 mg mL^{-1} Chl with 125 mM Tricine-KOH (pH 8.0). The Chl concentration was

[2] Mention of a trademark, propriety product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

measured spectrophotometrically in 80% acetone by measuring absorption at 647 and 664 nm according to the method of Ziegler and Egle (1965).

In some experiments, stromal fractions were separated from chloroplast membrane fractions by centrifuging lysed chloroplasts at 3000*g* at 4°C for 5 min. The supernatant was poured into Eppendorf tubes and centrifuged at 16,000*g* for 10 min. The supernatant from the second spin was considered the stromal fraction and stored on ice. The pellet from the first spin was resuspended in 30 mL of lysis buffer, centrifuged at 2000*g* at 4°C for 5 min, and resuspended in lysis buffer (in 0.10 M sorbitol) to a Chl concentration of 0.1 mg mL^{-1}. For these experiments BSA was excluded from the preparation so that protein concentrations of stromal fractions could be determined. Protein concentration was determined by the dye-binding method of Bradford (1976).

Preparation of Reconstituted Systems with Washed Thylakoids

Lysed chloroplasts were centrifuged at 2000*g* at 4°C for 5 min and the pellet was resuspended in 30 mL of lysis buffer after the supernatant was discarded. Centrifugation was repeated at 2000*g* at 4°C for 5 min and the pellet was resuspended to a concentration of 1 to 2 mg Chl mL^{-1} in lysis buffer containing 0.1 M sorbitol as an osmoticant. Maximum Rubisco activity of lysed chloroplasts was approximately 350 μmol CO_2 mg^{-1} Chl h^{-1} in most experiments. Residual Rubisco activity in washed thylakoid membranes ranged from 5 to 13%.

Rubisco Activation with Lysed Chloroplasts

Lysed chloroplasts were incubated in the dark at 25°C for 5 min, and then RuBP was added to a final concentration of 5 mM RuBP to inactivate Rubisco. After an additional 5 min in the dark, the activation buffer was added to provide final concentrations of 0.08 mg mL^{-1} Chl (lysed chloroplasts), 100 mM Tricine-KOH (pH 8.0), 10 mM $MgCl_2$, 1 mM ATP, 3 mM phosphocreatine, 20 units mL^{-1} creatine phosphokinase, and 1 mM $NaHCO_3$ (12.3 μM CO_2 at 25°C and pH 8.0). The activation mixture contained all components necessary to activate Rubisco-RuBP to Rubisco-CO_2-Mg^{2+} in the dark, but because of low CO_2, the final activity was less than observed in standard assays of Rubisco and reflects the equilibrium reached between Rubisco activation by Rubisco activase and the Rubisco deactivation from loss of activating CO_2 and Mg^{2+}. The time course of activation was followed at 25°C for 8 min in the dark or at 500 μmol photons m^{-2} s^{-1} by removing 50-μL aliquots at various times for Rubisco activity assays (see below). The light source was provided by projector lamp with the intensity reduced by a neutral density filter.

In experiments in which isolated stromal fractions were separated from chloroplast membrane fractions, stromal fractions were incubated in the dark for 5 min and in the presence of 5 mM RuBP for an additional 5 min. The activation buffer was added to chloroplast membrane fractions and reactions were initiated by addition of the stromal fractions.

Rubisco Activation with Reconstituted Systems

Washed thylakoid membranes of tomato were added to an activation buffer with final concentrations of 0.2 mg mL^{-1} washed thylakoid membranes, 100 mM Tricine-KOH (pH 8.0), 10 mM $MgCl_2$, 1 mM ATP, 4 mM RuBP, 3 mM phosphocreatine, 20 units mL^{-1} creatine phosphokinase, and 1 mM $NaHCO_3$. Rubisco activase (0.10 mg mL^{-1}) was added to the mixture. After 0.5 min, 0.5 mg mL^{-1} Rubisco-RuBP was added and Rubisco activation was followed at 25°C in the light or dark for 8 to 12 min by removing 50-μL aliquots at the designated times for Rubisco activity assays. In each experiment, Rubisco-RuBP and Rubisco activase from the same species, either spinach or tomato, were added to spinach or tomato thylakoid membranes. Rubisco-RuBP and Rubisco activase were purified and prepared as described by Salvucci et al. (1986a) and Robinson et al. (1988), respectively. Purified Rubisco was deactivated by filtration through Sephadex G-25 equilibrated with 20 mM Tricine-NaOH (pH 8.0) and 0.5 mM EDTA. After filtration, the inactive Rubisco-RuBP complex was formed by a 1-h incubation with 0.5 mM RuBP at 20°C.

Rubisco Activity Assays

Rubisco activity was assayed by removing 50-μL aliquots from the activation reactions at indicated times and adding to vials containing 100 mM Tricine-KOH (pH 8.0), 10 mM $NaH^{14}CO_3$ (0.5 Ci mol^{-1}), 10 mM $MgCl_2$, and 1.0 mM RuBP. Assays were terminated after 0.5 min with addition of 0.2 mL of 4 M HCOOH/1 M HCl.

Chemicals

RuBP was prepared enzymatically from Rib 5-P as described by Jordan and Ogren (1981) except that an ATP-regenerating system (1 mmol ATP, 10 mmol phosphocreatine, and 1000 units of creatine phosphokinase) was included in the reaction mixture to reduce the amount of ATP required in the preparation.

RESULTS

Following a 6-h exposure to 4°C at high light, the rate of CO_2 assimilation measured at 1000 and 350 μL L^{-1} CO_2 decreased by 56 and 61%, respectively, when compared to control leaves (Table I). The decrease in CO_2 assimilation caused by chilling was accompanied by a decline in both Rubisco activation state and RuBP levels in leaf extracts. After plants were allowed to recover for 24 h in the dark, the CO_2 assimilation rates measured at both high and atmospheric CO_2 were intermediate to those in leaves from control and chill treatments. However, Rubisco activation state and RuBP levels in leaf extracts remained low following the recovery period with values similar to those observed immediately following the chilling treatment. Rubisco activation state was not affected in plants chilled for 24 h at 4°C in the dark (data not shown).

Light stimulation of Rubisco activation was readily observed in lysed chloroplasts from unchilled plants (Fig. 1) but did not occur in lysed chloroplasts isolated from light-

Table I. *CO_2 assimilation rate, Rubisco activation state, and RuBP levels in tomato leaves in control, light-chilled, and recovered plants (n = 5, except "Chill" and "Recovery" CO_2 assimilation rates, where n = 3)*

Treatment	CO_2 Assimilation Rate		Rubisco Activity		Activation State	[RuBP]
	350 μL L^{-1} CO_2	1000 μL L^{-1} CO_2	Initial	Total		
	μmol CO_2 m^{-2} s^{-1}		*μmol CO_2 mg^{-1} protein min^{-1}*		%	*nmol mg^{-1} Chl*
Control	16.0 ± 1.4	26.4 ± 1.8	0.66 ± 0.08	0.92 ± 0.05	72 ± 11	165 ± 37
Chill	7.1 ± 0.7	10.2 ± 2.1	0.42 ± 0.04	0.80 ± 0.04	52 ± 6	69 ± 22
Recovery	11.5 ± 2.2	18.8 ± 0.4	0.51 ± 0.03	0.85 ± 0.05	60 ± 7	56 ± 42

chilled plants. For comparisons among these treatments, the endogenous Rubisco present in lysed chloroplasts and stromal fractions was inactivated by adding RuBP in the dark to form the inactive form of the enzyme, Rubisco-RuBP. In experiments in which stromal and chloroplast membrane fractions were recombined, light stimulation of Rubisco activation occurred when the stromal and membrane fractions isolated from control plants were recombined (Fig. 2A) and when the stromal fraction from chilled plants was mixed with membrane fractions from control plants (Fig. 2B). In contrast, when chloroplast membrane and stromal fractions from chilled plants were recombined or when chloroplast membranes were mixed with the stromal fraction from control plants, no light stimulation of Rubisco activation was observed (Fig. 2). These results show that chilling at high light influences the activation process through its effect on thylakoid membranes but not on the stromal enzymes Rubisco and Rubisco activase.

The effect of chilling in the light on Rubisco activation was reversible, since lysed chloroplasts isolated after a 24-h recovery period showed light stimulation of Rubisco activation (Fig. 3A) that was similar to activation by lysed chloroplasts from control plants (Fig. 1). Light stimulation was also apparent in an assay mixture reconstituted from stromal and membrane fractions isolated from recovered plants (Fig. 3B) but to a lesser degree than in control plants (Fig. 2).

The species specificity of the interaction between thylakoid membranes and Rubisco activase was investigated to determine whether the assay system could be simplified, since soluble enzymes were more readily obtained from spinach than from tomato leaves. Also, since tomato Rubisco is not readily activated by spinach Rubisco activase (Wang et al., 1992) and broken thylakoids are generally contaminated with Rubisco activity (Campbell and Ogren, 1992), background activity of the tomato thylakoids would be reduced if spinach Rubisco and Rubisco activase could be used in these experiments as the soluble phase in

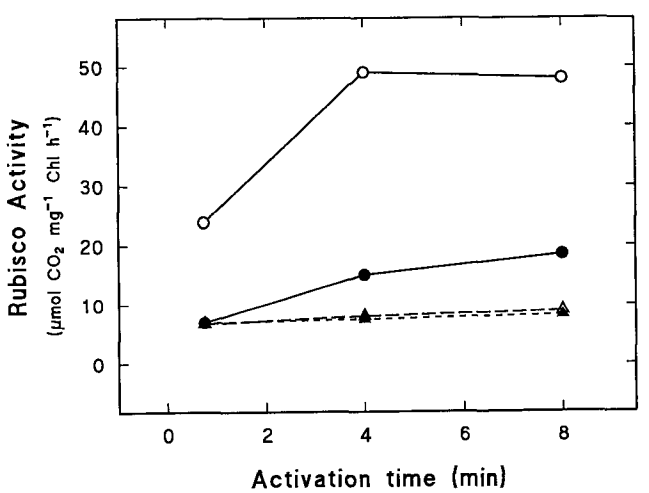

Figure 1. Chilling treatment inhibits light-stimulated Rubisco activation in lysed chloroplasts. Lysed chloroplasts were isolated from control (○, ●) and chilled (△, ▲) plants and Rubisco activation was measured in the dark (●, ▲) or at 500 μmol photons m^{-2} s^{-1} (○, △). Activation buffer was added and light was turned on at time zero. One of six typical experiments is presented.

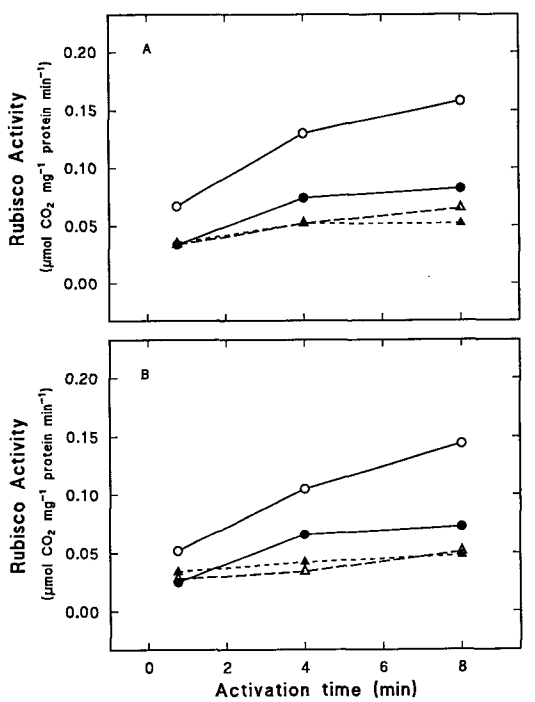

Figure 2. Reconstitution of chloroplasts by mixing stromal and membrane fractions from control and chilled plants. A, Rubisco activation in reconstituted chloroplasts in the light (○) and dark (●) and in reconstituted chloroplasts from chilled plants in the light (△) and dark (▲). B, Stimulation of Rubisco activation by membrane fractions from control (○, ●) and chilled plants (△, ▲) in the presence of stromal extracts from chilled plants in the light (○) and dark (●) and in the presence of stromal extracts from control plants in the light (△) and dark (▲). One of three typical experiments is presented.

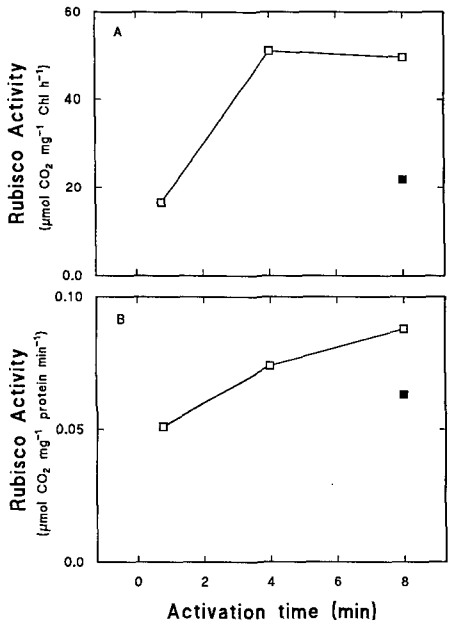

Figure 3. Rubisco activation in lysed and reconstituted chloroplasts from chilled plants after 24 h recovery at 25°C. A, Rubisco activation in lysed chloroplasts from recovered plants at 500 μmol photons m^{-2} s^{-1} (□) and in the dark (■). B, Rubisco activation in reconstituted chloroplasts from recovered plants in the light (□) and dark (■). One of three typical experiments is presented.

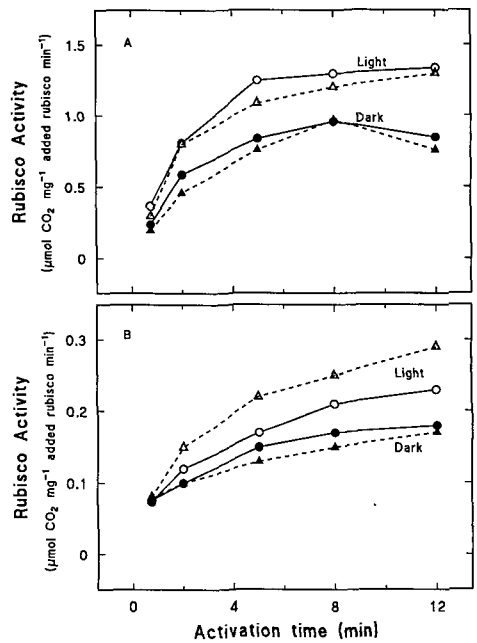

Figure 4. Interspecies reconstitution of chloroplasts. A, Stimulation of spinach Rubisco activity by spinach (○, ●) and tomato (△, ▲) thylakoids in the light (○, △) and dark (●, ▲). B, Stimulation of tomato Rubisco activity by spinach (○, ●) and tomato (△, ▲) thylakoids in the light (○, △) and dark (●, ▲). One of three typical experiments is presented.

reconstituted chloroplasts. We found that both spinach and tomato thylakoids effectively activated spinach Rubisco in the presence of spinach Rubisco activase (Fig. 4A). Spinach thylakoids also stimulated tomato Rubisco in the presence of tomato Rubisco activase, although activation was less than observed with tomato thylakoids (Fig. 4B). Utilizing the interspecies reconstituted system corroborated the effect of chilling on tomato membranes in activating Rubisco. Spinach Rubisco activity was stimulated by control tomato thylakoids in the light, and this activation was considerably reduced in the presence of thylakoids from chilled tomato plants (Fig. 5). Rubisco-stimulating activity was largely restored in thylakoid membranes from chilled plants after 24 h of recovery at 25°C (Fig. 5).

DISCUSSION

The prolonged inhibition of photosynthesis following exposures to low temperature at high light in tomato involves more than a single limiting factor since both the Rubisco activation state and RuBP levels were reduced after chilling (Table I). Sassenrath et al. (1990) found that the decrease in RuBP levels following chilling in the light was due most likely to lower turnover of FBP and SBP, resulting from a substantial reduction in the activities of the stromal bisphosphatases, Fru-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase. Thus, an important consequence of chilling in the light appears to be the disruption of RuBP regeneration via thioredoxin-dependent activation of the stromal bisphosphatases. Our results con-

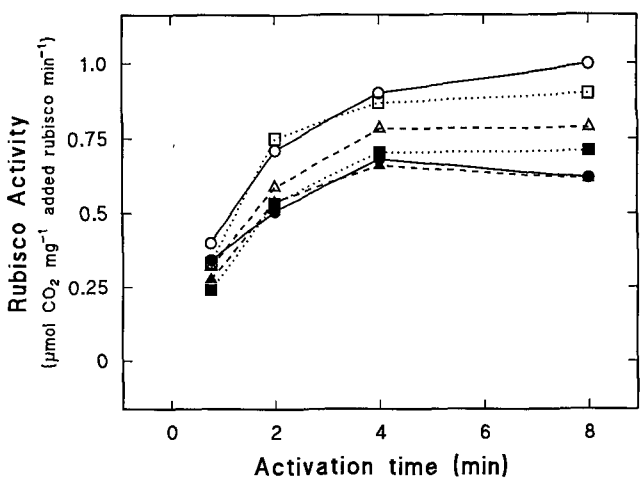

Figure 5. Light stimulation of Rubisco activation in reconstituted systems including spinach Rubisco-RuBP (0.50 mg mL^{-1}), spinach Rubisco activase (0.10 mg mL^{-1}), 4 mM RuBP, 1 mM ATP, and an ATP-regenerating system (see text) and washed thylakoid membranes from tomato plants of control (○, ●), chilled (△, ▲), or recovery (□, ■) treatments. Rubisco activation was begun at time zero with the addition of spinach Rubisco-RuBP in the light (○, △, □) or dark (●, ▲, ■). One of four typical experiments is presented.

firm a limitation in photosynthetic RuBP regeneration but also demonstrate that the light-dependent stimulation of Rubisco activation is inhibited following chilling treatment.

Several components could be affected by the imposed chilling regime when considering the basis for the reduced Rubisco activity observed in our experiments. First, there is a need to distinguish between direct effects on catalytic turnover of the enzyme complex and the process of activation. Loss of Rubisco activity that occurs at low temperatures was readily reversible by rewarming both in vitro (Kawashima et al., 1971; Chollet and Anderson, 1977) and in vivo (Sassenrath and Ort, 1990). Our results indicate that the catalytic activities of both Rubisco and Rubisco activase are unaffected by short-term chilling in the light (Fig. 2B). In contrast, long-term (2 weeks) chilling of tomato plants led to an irreversible decrease in Rubisco activity (Brüggemann et al., 1992).

The activation of Rubisco by Rubisco activase requires ATP, an appropriate pH, Mg^{2+}, and CO_2, and also the light-mediated association or interaction of Rubisco activase with thylakoid membranes (Campbell and Ogren, 1992). Rubisco activation also requires electron transport through PSI and the presence of a transthylakoid pH difference (Campbell and Ogren, 1990b). The most dramatic effect of chilling in our experiments was a greatly reduced ability of thylakoid membranes to stimulate Rubisco activation in the light, indicating that chilling affects this important regulatory step in the CO_2 fixation process in tomato. Rubisco activation state represents an equilibrium between Rubisco activation by the Rubisco activase system and deactivation by RuBP or other sugar phosphates (Salvucci et al., 1986b); therefore, deactivated enzyme species would be favored by defects in the light-induced stimulation of Rubisco activity. The lower Rubisco activation state that we observed in extracts from light-chilled leaves was caused by a deficiency in the Rubisco activase system. Specifically, the data suggest that an impaired association between thylakoid membranes and Rubisco activase reduces CO_2 assimilation and indicate that Rubisco inactivation at low temperatures is an important step in the events leading to the chilling-induced inhibition of photosynthesis. The reduced RuBP levels found in light-chilled tomato leaves likely moderates, but does not prevent, the shift in equilibrium toward Rubisco deactivation.

The association between Rubisco activase and thylakoid membranes appears to be relatively nonspecific, since the activation of both spinach and tomato Rubisco were stimulated by tomato thylakoids in the light. This nonspecificity is in sharp contrast to the poor ability of spinach Rubisco activase to activate Rubisco from tomato and other Solanaceae species (Wang et al., 1992). Thus, the association between Rubisco activase and thylakoid membranes is distinct from specific Rubisco/Rubisco activase interactions.

In conclusion, persistent photosynthetic inhibition that follows chilling at high light in tomato is due to factors affecting both RuBP levels and Rubisco activation. With respect to Rubisco activation, light stimulation is temporarily lost by effects of chilling on thylakoid membranes but not on the activity of either Rubisco or Rubisco activase. This loss of interaction between thylakoid membranes and Rubisco activation represents a key step in the events leading to photosynthetic inhibition following chilling in the light.

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